

CLAIMS

WHAT IS CLAIMED IS:

1. A method of producing transformed plant cells, the method comprising:
culturing at least one non-apical meristemic cell to produce one or more
5 organogenic cells; and,
introducing at least one nucleic acid segment into the organogenic cells
to produce one or more transformed organogenic cells.
2. The method of claim 1, wherein the non-apical meristemic cell is derived
from a monocotyledonous plant.
- 10 3. The method of claim 1, wherein the non-apical meristemic cell is derived
from a dicotyledonous plant.
4. The method of claim 1, wherein the non-apical meristemic cell is derived
from a plant selected from the genera: *Ananas*, *Musa*, *Vitis*, *Fragaria*, *Lotus*, *Medicago*,
Onobrychis, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Carica*, *Persea*, *Prunus*, *Syrgrus*,
15 *Theobroma*, *Coffea*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*,
Raphanus, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*,
Solanum, *Petunia*, *Digitalis*, *Majorana*, *Mangifera*, *Cichorium*, *Helianthus*, *Lactuca*,
Bromus, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesia*, *Pelargonium*, *Panicum*,
Pennisetum, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucurbita*, *Cucumis*, *Browaalia*,
20 *Lolium*, *Malus*, *Apium*, *Gossypium*, *Vicia*, *Lathyrus*, *Lupinus*, *Pachyrhizus*, *Wisteria*,
Stizolobium, *Agrostis*, *Phleum*, *Dactylis*, *Sorgum*, *Setaria*, *Zea*, *Oryza*, *Triticum*, *Secale*,
Avena, *Hordeum*, *Saccharum*, *Poa*, *Festuca*, *Stenotaphrum*, *Cynodon*, *Coix*, *Olyrae*,
Phareae, *Glycine*, *Pisum*, *Psidium*, *Passiflora*, *Cicer*, *Phaseolus*, *Lens*, and *Arachis*.
5. The method of claim 1, wherein the non-apical meristemic cell comprises a
25 pineapple cell selected from the group consisting of: a Smooth Cayenne cell, a Red
Spanish cell, a Perolera cell, a Pernambuco cell, and a Primavera cell.
6. The method of claim 1, wherein the nucleic acid segment confers resistance
to the transformed organogenic cells from one or more of: insects, drought, nematodes,
viral disease, bacterial disease, or herbicides.

7. The method of claim 1, wherein the nucleic acid segment stably integrates into the genome of the transformed organogenic cells.

8. The method of claim 1, wherein the nucleic acid segment comprises a selectable marker.

5 9. The method of claim 1, wherein the nucleic acid segment comprises or encodes one or more of: an ACC synthase, an ACC oxidase, a malic enzyme, a malic dehydrogenase, a glucose oxidase, a chitinase, a defensin, an expansin, a hemicellulase, a xyloglucan transglycosylase, an apetala gene, a leafy gene, a knotted-related gene, a homeobox gene, an Etr-related gene, or a ribonuclease.

10 10. The method of claim 1, wherein the nucleic acid segment comprises at least one sense nucleic acid segment that corresponds to at least a portion of at least one endogenous gene.

15 11. The method of claim 1, wherein the nucleic acid segment comprises at least one sense nucleic acid segment that corresponds to at least a portion of at least one exogenous gene.

12. The method of claim 1, wherein the nucleic acid segment comprises at least one antisense nucleic acid segment that corresponds to at least a portion of at least one endogenous gene.

20 13. The method of claim 1, wherein the nucleic acid segment encodes at least one polypeptide transcription factor. /

14. The method of claim 1, wherein the nucleic acid segment encodes at least one promoter and/or at least one enhancer, which nucleic acid segment homologously recombines with at least one promoter and/or at least one enhancer of at least one endogenous gene.

25 15. The method of claim 1, wherein the nucleic acid segment is introduced into the organogenic cells using Agrobacterium-mediated delivery.

30 16. The method of claim 1, wherein the nucleic acid segment is introduced into the organogenic cells using at least one nucleic acid delivery technique selected from the group consisting of: pollen-mediated delivery, direct nucleic acid transfer to at least one protoplast of the organogenic cells, microprojectile bombardment, microinjection,

macroinjection of inflorescence, whisker-mediated impregnation, laser perforation, and ultrasonification.

17. The method of claim 1, further comprising:

generating at least one plant from the transformed organogenic cells.

5 18. The method of claim 1, wherein the nucleic acid segment encodes a polypeptide.

19. The method of claim 18, wherein the polypeptide comprises a plant polypeptide.

20. The method of claim 18, wherein the polypeptide is artificially evolved.

10 21. The method of claim 18, wherein the polypeptide is heterologous to the organogenic cells.

22. The method of claim 18, wherein the polypeptide is homologous to at least one endogenous polypeptide of the organogenic cells.

15 23. The method of claim 18, wherein the polypeptide comprises at least one carotenoid biosynthetic polypeptide that is selected from the group consisting of: an isopentenyl diphosphate isomerase, a geranylgeranyl pyrophosphate synthase, a phytoene synthase, a phytoene desaturase, a ζ -carotene desaturase, a lycopene β -cyclase, a lycopene ε -cyclase, a β -carotene hydroxylase, and an ε -hydroxylase.

20 24. The method of claim 18, wherein the nucleic acid segment is operably linked to a constitutive promoter.

25. The method of claim 18, wherein the nucleic acid segment is operably linked to an inducible promoter.

26. A method of producing transformed plant cells, the method comprising:
culturing at least one meristemic cell to produce at least one shoot;
25 culturing at least one explant from the shoot to produce one or more organogenic cells; and,
introducing at least one nucleic acid segment into the organogenic cells
to produce one or more transformed organogenic cells.

27. The method of claim 26, wherein the explant comprises one or more non-apical meristemic cells.

28. The method of claim 26, wherein the meristemic cell is derived from a monocotyledonous plant.

5 29. The method of claim 26, wherein the meristemic cell is derived from a dicotyledonous plant.

30. The method of claim 26, wherein the meristemic cell is derived from a plant selected from the genera: *Ananas*, *Musa*, *Vitis*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Carica*, *Persea*, *Prunus*, *Syragrus*,
10 *Theobroma*, *Coffea*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Mangifera*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucurbita*, *Cucumis*, *Browaalia*,
15 *Lolium*, *Malus*, *Apium*, *Gossypium*, *Vicia*, *Lathyrus*, *Lupinus*, *Pachyrhizus*, *Wisteria*, *Stizolobium*, *Agrostis*, *Phleum*, *Dactylis*, *Sorgum*, *Setaria*, *Zea*, *Oryza*, *Triticum*, *Secale*, *Avena*, *Hordeum*, *Saccharum*, *Poa*, *Festuca*, *Stenotaphrum*, *Cynodon*, *Coix*, *Olyraeae*, *Phareae*, *Glycine*, *Pisum*, *Psidium*, *Passiflora*, *Cicer*, *Phaseolus*, *Lens*, and *Arachis*.

20 31. The method of claim 26, wherein the meristemic cell comprises a pineapple cell selected from the group consisting of: a Smooth Cayenne cell, a Red Spanish cell, a Perolera cell, a Pernambuco cell, and a Primavera cell.

32. The method of claim 26, wherein the nucleic acid segment confers resistance to the transformed organogenic cells from one or more of: insects, drought, nematodes, viral disease, bacterial disease, or herbicides.

25 33. The method of claim 26, wherein the nucleic acid segment stably integrates into the genome of the transformed organogenic cells.

34. The method of claim 26, wherein the nucleic acid segment comprises a selectable marker.

30 35. The method of claim 26, wherein the nucleic acid segment comprises or encodes one or more of: an ACC synthase, an ACC oxidase, a malic enzyme, a malic

dehydrogenase, a glucose oxidase, a chitinase, a defensin, an expansin, a hemicellulase, a xyloglucan transglycosylase, an apetala gene, a leafy gene, a knotted-related gene, a homeobox gene, an Etr-related gene, or a ribonuclease.

36. The method of claim 26, wherein the nucleic acid segment comprises at least one sense nucleic acid segment that corresponds to at least a portion of at least one endogenous gene.

37. The method of claim 26, wherein the nucleic acid segment comprises at least one sense nucleic acid segment that corresponds to at least a portion of at least one exogenous gene.

38. The method of claim 26, wherein the nucleic acid segment comprises at least one antisense nucleic acid segment that corresponds to at least a portion of at least one endogenous gene.

39. The method of claim 26, wherein the nucleic acid segment encodes at least one polypeptide transcription factor.

40. The method of claim 26, wherein the nucleic acid segment encodes at least one promoter and/or at least one enhancer, which nucleic acid segment homologously recombines with at least one promoter and/or at least one enhancer of at least one endogenous gene.

41. The method of claim 26, wherein the nucleic acid segment is introduced into the organogenic cells using Agrobacterium-mediated delivery.

42. The method of claim 26, wherein the nucleic acid segment is introduced into the organogenic cells using at least one nucleic acid delivery technique selected from the group consisting of: pollen-mediated delivery, direct nucleic acid transfer to at least one protoplast of the organogenic cells, microprojectile bombardment, microinjection, macroinjection of inflorescence, whisker-mediated impregnation, laser perforation, and ultrasonification.

43. The method of claim 26, further comprising:
generating at least one plant from the transformed organogenic cells.

44. The method of claim 26, wherein the meristemic cell is derived from a core and/or a stem of a crown of a pineapple plant or a leaf base of pineapple plant.

45. The method of claim 44, wherein the meristemic cell is a lateral meristemic cell or a meristem cell induced by tissue culture.

46. The method of claim 44, wherein the meristemic cell is a crown tip meristemic cell.

5 47. The method of claim 26, wherein the nucleic acid segment encodes a polypeptide.

48. The method of claim 47, wherein the polypeptide comprises a plant polypeptide.

49. The method of claim 47, wherein the polypeptide is artificially evolved.

10 50. The method of claim 47, wherein the polypeptide is heterologous to the organogenic cells.

51. The method of claim 47, wherein the polypeptide is homologous to at least one endogenous polypeptide of the organogenic cells.

15 52. The method of claim 47, wherein the polypeptide comprises at least one carotenoid biosynthetic polypeptide that is selected from the group consisting of: an isopentenyl diphosphate isomerase, a geranylgeranyl pyrophosphate synthase, a phytoene synthase, a phytoene desaturase, a ζ -carotene desaturase, a lycopene β -cyclase, a lycopene ϵ -cyclase, a β -carotene hydroxylase, and an ϵ -hydroxylase.

20 53. The method of claim 47, wherein the nucleic acid segment is operably linked to a constitutive promoter.

54. The method of claim 47, wherein the nucleic acid segment is operably linked to an inducible promoter.